

CLAIMS

1. Vector for transferring nucleic acids, characterized in that it comprises a double-stranded  
5 DNA molecule and at least one oligonucleotide which is coupled to a targeting signal and which is capable of forming a triple helix with a specific sequence present on the said double-stranded DNA molecule.

2. Vector for transferring nucleic acids  
10 according to claim 1, characterized in that the double-stranded DNA molecule is a plasmid or an episome.

3. Vector for transferring nucleic acids according to claim 2, characterized in that the double-stranded DNA molecule is a plasmid in circular form or  
15 in a supercoiled state.

4. Vector for transferring nucleic acids according to claims 1 to 3, characterized in that the double-stranded DNA molecule comprises an expression cassette consisting of one or more genes of interest  
20 under the control of one or more promoters or of a transcriptional terminator which is active in mammalian cells.

5. Vector for transferring nucleic acids according to claim 4, characterized in that the gene of  
25 interest is a nucleic acid encoding a therapeutic product.

6. Vector for transferring nucleic acids

according to one of claims 1 to 5, characterized in that the specific sequence present on the double-stranded DNA molecule is a homopurine-homopyrimidine sequence.

5                   7.    Vector for transferring nucleic acids according to one of claims 1 to 6, characterized in that the specific sequence present on the double-stranded DNA molecule is a sequence which is naturally present on the double-stranded DNA or a synthetic or a  
10 natural sequence which is introduced artificially into the double-stranded DNA.

                  8.    Vector for transferring nucleic acids according to one of claims 1 to 7, characterized in that the oligonucleotide comprises a sequence poly-CTT  
15 and the specific sequence present on the double-stranded DNA molecule is a sequence poly-GAA.

                  9.    Vector for transferring nucleic acids according to one of claims 1 to 7, characterized in that the oligonucleotide comprises the sequence  
20 GAGGCTTCTTCTTCTTCTTCTTCTT (SEQ ID No. 1) or the sequence (CTT)<sub>7</sub> (SEQ ID No. 2).

                  10.   Vector for transferring nucleic acids according to one of claims 1 to 7, characterized in that the specific sequence present on the double-  
25 stranded DNA molecule comprises the sequence 5'-AAGGGAGGGAGGAGAGGAA-3' (SEQ ID No. 3) and the oligonucleotide comprises the sequence

5'-AAGGAGAGGAGGGAGGGAA-3' (SEQ ID No. 4) or

5'-TTGGTGTGGTGGGTGGGTT-3' (SEQ ID No. 5).

11. Vector for transferring nucleic acids according to one of claims 1 to 7, characterized in that the specific sequence present on the double-stranded DNA molecule comprises all or part of the sequence 5'-CTTCCCGAAGGGAGAAAGG-3' (SEQ ID No. 6) present in the replication origin ColE1 of E. coli, and the oligonucleotide possesses the sequence

10 5'-GAAGGGTTCTTCCTCTTTCC-3' (SEQ ID No. 7).

12. Vector for transferring nucleic acids according to one of claims 1 to 7, characterized in that the specific sequence present on the double-stranded DNA molecule comprises the sequence

15 5'-GAAAAAGGAAGAG-3' (SEQ ID No. 8) or the sequence 5'-AAAAAAGGGAATAAGGG-3' (SEQ ID No. 10) present in the  $\beta$ -lactamase gene of the plasmid pBR322 and of E. Coli, respectively.

13. Vector for transferring nucleic acids

20 according to one of claims 1 to 7, characterized in that the specific sequence present on the double-stranded DNA molecule comprises the sequence 5'-AAGAAAAAAAAGAA-3' (SEQ ID NO. 9) present in the replication origin  $\gamma$  of the plasmids containing a

25 conditional replication origin such as pCOR.

14. Vector for transferring nucleic acids according to one of claims 1 to 13, characterized in

that the oligonucleotide comprises at least 3 base pairs.

15. Vector for transferring nucleic acids according to claim 14, characterized in that the  
5 oligonucleotide comprises 5 to 30 base pairs.

16. Vector for transferring nucleic acids according to one of claims 1 to 15, characterized in that the oligonucleotide exhibits at least one chemical modification making it resistant to, or protecting it  
10 from, nucleases, or increasing its affinity towards the specific sequence present on the double-stranded DNA molecule.

17. Vector for transferring nucleic acids according to one of claims 1 to 16, characterized in  
15 that the oligonucleotide is a succession of nucleosides which have undergone modification of the backbone.

18. Vector for transferring nucleic acids according to one of claims 1 to 16, characterized in that the oligonucleotide is coupled to an alkylating  
20 agent forming a covalent bond at the level of the bases of the double-stranded DNA.

19. Vector for transferring nucleic acids according to claim 18, characterized in that the said alkylating agent is photoactivable.

25 20. Vector for transferring nucleic acids according to claims 18 and 19, characterized in that the said alkylating agent is a psoralen.

21. Vector for transferring nucleic acids according to claims 1 to 20, characterized in that the targeting signal interacts with a component of the extracellular matrix, a plasma membrane receptor, targets an intracellular compartment, and/or improves the intracellular flow of the double-stranded DNA.

22. Vector for transferring nucleic acids according to claim 21, characterized in that the said targeting signal comprises growth factors (EGF, PDGF, TGF $\beta$ , NGF, IGF, I, FGF), cytokines (IL-1, IL-2, TNF, Interferon, CSF), hormones (insulin, growth hormone, prolactin, glucagon, thyroid hormone, steroid hormones), sugars which recognize lectins, immunoglobulins, transferrin, lipoproteins, vitamins such as vitamin B12, peptide or neuropeptide hormones (tachykinins, neurotensin, VIP, endothelin, CGRP, CCK, and the like), or any unit recognized by the integrins, for example the peptide RGD, or by other extrinsic proteins of the cell membrane.

23. Vector for transferring nucleic acids according to claim 21, characterized in that the targeting signal is an intracellular targeting signal, such as a nuclear homing sequence (NLS).

24. Vector for transferring nucleic acids according to claim 23, characterized in that the nuclear homing signal is the NLS sequence of the SV40 T antigen.

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25. Vector for transferring nucleic acids according to claim 21, characterized in that the targeting signal allows both extracellular targeting and intracellular targeting.

5           26. Vector for transferring nucleic acids according to claims 1 to 25, characterized in that the coupling of the targeting signal to the oligonucleotide is obtained by synthesis on a solid phase or in solution, in particular by establishing disulphide,  
10 thioether, ester, amide or amine bonds.

27. Composition, characterized in that it contains at least one vector as defined in one of claims 1 to 26.

28. Composition according to claim 27,  
15 characterized in that it contains, in addition, one or more transfecting agents.

29. Composition according to claim 28, characterized in that the transfecting agent is a cationic lipid, a lipopolyamine or a cationic polymer.

20           30. Composition according to claims 27 to 29, characterized in that it contains, in addition, one or more adjuvants capable of combining with the vector as defined in one of claims 1 to 24/transfecting agent complexes.

25           31. Composition according to claim 30, characterized in that the adjuvant is one or more neutral lipids chosen from natural or synthetic lipids

which are zwitterionic or which lack ionic charge under physiological conditions.

32. Composition according to either of claims 30 and 31, characterized in that the neutral  
5 lipid(s) is(are) chosen from lipids containing two fatty chains.

33. Composition according to claims 30 to 32, characterized in that the neutral lipid(s) is (are) chosen from dioleoylphosphatidylethanolamine (DOPE),  
10 oleoylpalmitoylphosphatidylethanolamine (POPE), di-stearoyl, -palmitoyl, -mirystoylphosphatidyl-ethanolamines as well as their derivatives which are N-methylated 1 to 3 times, phosphatidylglycerols, diacylglycerols, glycosyldiacylglycerols, cerebrosides  
15 (such as in particular galactocerebrosides), sphingolipids (such as in particular sphingomyelins) or asialogangliosides (such as in particular asialoGM1 and GM2).

34. Composition according to claim 30,  
20 characterized in that the adjuvant is or comprises a compound which is involved in the condensation of the DNA.

35. Composition according to claim 34, characterized in that the said compound is derived, as  
25 a whole or in part, from a histon, a nucleolin and/or a protamine, or consists, as a whole or in part, of peptide units (KTPKKAKKP SEQ ID No.16) and/or (ATPAKKAA

SEQ ID No.17) repeated continuously or otherwise, it being possible for the number of units to vary between 2 and 10.

36. Composition according to any one of  
5 claims 27 to 35, characterized in that it comprises a pharmaceutically acceptable vehicle for an injectable formulation.

37. Composition according to any one of  
10 claims 27 to 35, characterized in that it comprises a pharmaceutically acceptable vehicle for application to the skin and/or to the mucous membranes.

38. Use of a vector for transferring nucleic acids as defined in one of claims 1 to 26 for the manufacture of a medicament intended to treat diseases.

15 39. Method of transfecting nucleic acids into cells, characterized in that it comprises the following steps:

(1) synthesis of the oligonucleotide-targeting signal chimera,

20 (2) bringing the chimera synthesized in (1) into contact with a double-stranded DNA so as to form triple helices,

(3) optionally, complexing the vector obtained in (2) with one or more transfection agents  
25 and/or one or more adjuvants, and

(4) bringing the cells into contact with the complex formed in (2) or, if applicable, in (3).



40. Method of treating diseases by  
administration of a vector for transferring nucleic  
acids as defined in any one of Claims 1 to 26  
containing a double-stranded DNA capable of correcting  
5 the said disease.

41. Recombinant cell containing a nucleic  
acid transfer vector as defined in any one of claims 1  
to 26.

42. Recombinant cell according to claim 41,  
10 characterized in that it is a eukaryotic cell.